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(71)(72) Applicant and Inventor: WILKINS, Ebtisam, S. [US/US]; 9961 Florence Avenue N.E., Albuquerque, NM 87122 (US).

(74) Agents: BECKER, Robert, W. et al.; Suite B, 11896 N. Highway 14, Tijeras, NM 87059 (US).

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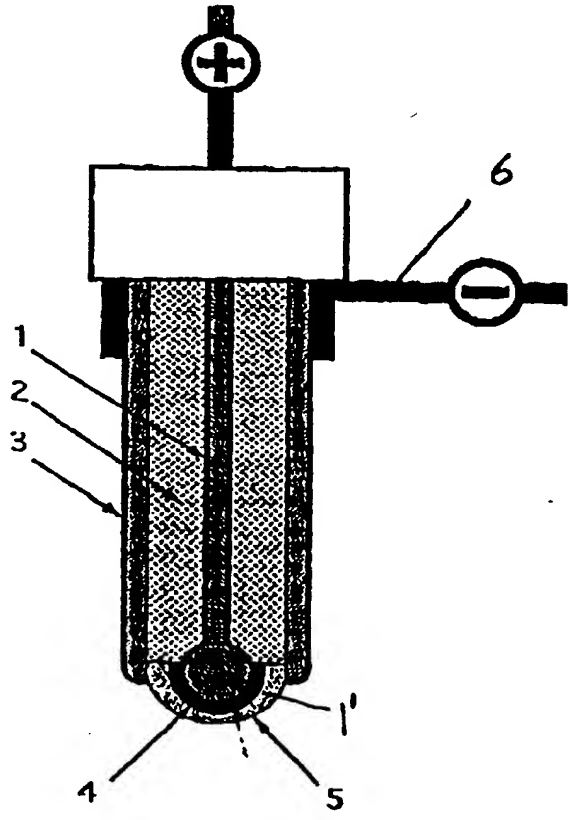
(57) Abstract

A coated wire sensor, including a platinum wire (1) as a working electrode, a sensor body (3) in the form of a hollow member disposed about at least a portion of the platinum wire, the sensor body being spaced from the platinum wire, a reference electrode (6) that is operatively associated with a first end of the platinum wire, but is not in contact with the wire, insulation (2) disposed along at least a portion of a length of the platinum wire between the wire and the sensor body and/or the reference electrode, enzyme-containing material (4) disposed on the first end of the platinum wirer, the enzyme-containing material comprising enzyme chemically linked to fine carbon particles, and a coating (5) disposed over the enzyme-containing material.



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COATED WIRE SENSOR

Technical Field

The present invention relates to a coated wire sensor, which can be used in the laboratory, has clinical applications, has applications in the food and pharmaceutical industries, and in particular can be used in the management of hemorrhagic shock.

Among other uses, a coated wire sensor can be used to increase the survival rate of injured patients who are at risk of hemorrhagic shock, especially while they are being transported to a hospital. The important point in such an application is that unlike diabetes, hemorrhagic shock requires direct intravascular implantation of such sensors, and the monitoring of glucose levels for up to several hours.

For example, hemorrhagic shock is characterized by the alteration of the glucose concentrations between the hypoglycemic and hyperglycemic levels. Hence, a linearity of the sensor signal versus the glucose concentration is required. This linearity should be at least 22 mM (400 mg/dL) to assure linear output of the sensor within a safety margin. Unfortunately, the heretofore known devices do not provide for a high enough linearity.

It is therefore an object of the present invention to provide a coated wire sensor that has a linear range of at least 22 mM, as well as a short response time and as small an effect of common physiological and industrial fermentation process interference on the sensor signal as possible. Furthermore, the sensor should also have a lifetime of at least five days with no

significant change in the sensor signal during this time period.

Background Art

Enzyme Entrapped in Conducting Polymers

Electropolymerization involves the electrochemical oxidation of a monomer from a solution containing the enzyme to form a conducting or non-conducting polymer on the electrode surface. This approach has several advantages. First, this process can be governed by the electrode potential, and therefore allows accurate control of the polymer film thickness and hence the amount of entrapped enzyme. Second, since the polymerization occurs locally at the electrode surface, it can be used to confine an enzyme precisely at the electrode without cross-immobilizing it on a neighboring electrode. This property is suitable for the fabrication of micro-arrays. Third, it is possible to use this technique for building multilayer structures, either one or more enzymes layered within a single polymer, or one enzyme within a multilayered copolymer.

Foulds and Lowe (N.C. Foulds and C.R. Lowe, J. Chem. Soc., Faraday Trans., 82 (1986) 1259) and Umana and Waller (M. Umana and J. Waller, Anal. Chem., 58 (1986) 2979) were the first to demonstrate the possibility of employing GOD (glucose oxidose) entrapped in poly(pyrrole) in the fabrication of amperometric glucose sensors. Since then, a large amount of research effort has been directed towards the development of glucose sensors employing this concept (P.N. Bartlett and J.M. Cooper, J. Electroanal. Chem., 362 (1993) 1). Fortier et al. investigated and

reported on the concentrations of pyrrole and GOD which led to optimum sensor response (G. Fortier, E. Brassard and D. Belanger, Biosensors and Biolectronics, 5 (1990) 473). They reported that these optimum conditions led to a biosensor with a high enzyme loading and a longer lifetime.

There have been other approaches to the fabrication of pyrrole sensors besides physical entrapment of GOD. Covalent electropolymerization of GOD to pyrrole by modifying different side chains on the enzyme has been reported (S.E. Wolawacz, B.F. Yon-Hin and C.R. Lowe, Anal. Chem., 64 (1992) 1541). The modification reactions involved carbodiimide coupling or Schiff base formation (B.F. Yon-Hin, M. Smolander, T. Crompton and C.R. Lowe, Anal. Chem., 65 (1993) 2067). Foulds et al also demonstrated the possible use of covalently attached ferrocenes to pyrrole monomers as a step towards the fabrication of reagentless sensors (N.C. Foulds and C.R. Lowe, Anal. Chem., 60 (1988) 2473). There have also been other reports on incorporating mediators in pyrrole films as anionic counter ions (Y. Kajiya, H. Sugai, C. Iwakura and H. Yoneyama, Anal. Chem., 63 (1991 49; S. Yabuki, H. Shinohara, Y. Ikariyama and M. Aizawa, J. Electroanal. Chem. 277 (1990) 179).

Although most of the research has focused on poly(pyrrole) sensors, there have been reports of using other conducting polymers such as poly(N-methylpyrrole) (P.N. Bartlett and P.G. Whittaker, J. Electroanal. Chem., 224 (1987) 37), poly(aniline) J.C. Cooper and E.A.H. Hall, Biosensors, 7 (1992) 473).

Enzyme Entrapped in Non-Conducting Polymers

Non-conducting polymers have been studied as they have several advantageous characteristics not found in the conducting polymers. First, their growth is self limiting, thus producing thin polymer films. This results in a short sensor response time. The thin film also allows a higher enzyme content leading to a large sensor response and a high sensitivity. Non-conducting polymers also have a characteristic permselectivity, greatly decreasing the effect of common physiological interferents on the sensor response.

A non-conducting polymer that has been the focus of much research is 1,2-phenylenediamine.

Yacynych et al. were the first to demonstrate that the oxidation of 1,2-phenylenediamine is irreversible and formed an insulating polymer film completely covering the electrode surface (A.M. Yacynych and H.B. Mark, J. Electrochem. Soc., 123 (1976) 1346). Heineman et al demonstrated that 1,2-phenylenediamine forms a polymeric film over the pH range from 4 to 10 (W.R. Heineman, H.J. Wick, and A.M. Yacynych, Anal. Chem., 52 (1980) 345). After the possibility of entrapping GOD in these non-conducting polymer films had been demonstrated, numerous studies have appeared applying this concept in the fabrication of glucose sensors.

Sasso et al. proposed a glucose sensor having a platinized reticulated vitreous carbon electrode with GOD entrapped in a poly(1,2-phenylenediamine) film (S.V. Sasso, R.J. Pierce, R. Walla, and A.M.

Yacynych, Anal. Chem., 62 (1990) 1111). Their sensor was used in Flow Injection Analysis for determining glucose levels in human serum. They demonstrated the ability of the poly(1,2-phenylenediamine) film in increasing the thermal stability of the GOD enzyme. Malitesta et al. constructed a glucose sensor, employing the same principle, but using platinum electrodes (C. Malitesta, F. Palmisano, L. Torsi and P.G. Zambonin, Anal. Chem., 62 (1990) 2735). Their sensor had a response time of 1 second and demonstrated almost complete rejection of common physiological interferents, especially ascorbate. Wang et al. used a similar construction but with an additional outer lipid layer (J. Wang and H. Wu, Anal. Chim. Acta, 283 (1993) 683). Their sensor showed a response time of 8 to 10 seconds. They demonstrated that the extra lipid layer almost completely eliminated the sensor response to acetaminophen (another common physiological interferent).

Another non-conducting polymer that has been studied is poly(1,3-phenylenediamine). This polymer is not commonly used in the construction of glucose sensors although it possesses the same permselective properties as poly(1,2-phenylenediamine). Geise et al. have demonstrated the use of 1,1'-dimethylferrocene as a mediator entrapped with GOD in a poly(1,3-phenylenediamine) film (R.J. Geise, S.Y. Roach and A.M. Yacynych, Anal. Chim. Acta, 281 (1993) 467). They report that this combination results in a sensor with a linear range of more than 22 mM and a lifetime of four months. Yacynych et al. have also used poly(1,3-phenylenediamine)

for the fabrication of miniaturized glucose sensors (A.M. Yacynych, and E.R. Reynolds, Third World Congress: Biosensors '94, 1-3 June 1994, Proceedings). They report their sensors to have a response time of 40 seconds and decreased fouling at the sensor surface (see also Yacynych et al, U.S. Patent 5,286,364).

There are reports on the use of other non-conducting polymers, such as poly-(phenol) (J. Wang, S-P Chen and M-S Lin, J. Electroanal. Chem., 273 (1989) 231; R.L. McClarley, E.A. Irene and R.W. Murray, J. Phys. Chem., 95 (1991) 2492; P.N. Bartlett and D.J. Caruana, Analyst, 117 (1992) 1287). Poly(phenol) has not been used as extensively as poly(phenylenediamine), probably because of its oxidative properties if it comes into contact with air and oxygen and its light sensitivity. Poly(indole) [53] has also been used in the fabrication of glucose biosensors.

Brief Description of the Drawings

These and other objects and advantages of the present invention will appear more clearly from the following specification in conjunction with the accompanying schematic drawings, in which:

Fig. 1 is a cross-sectional view of a first exemplary embodiment of the inventive coated wire sensor;

Fig. 2 is a cross-sectional view of a second exemplary embodiment of an inventive coated wire sensor; and

Fig. 3 shows a third exemplary embodiment of an inventive coated wire sensor.

Disclosure of the Invention

The coated wire sensor of the present

invention is characterized primarily by: a platinum wire as a working electrode, a sensor body in the form of a hollow member disposed about at least a portion of the platinum wire, with the sensor body being spaced from the wire, a reference electrode that is operatively associated with a first end of the platinum wire but is not in contact with the wire, insulation disposed along at least a portion of the length of the platinum wire between the wire and the sensor body and/or the reference electrode, enzyme-containing material disposed on the first end of the platinum wire, with this enzyme-containing material comprising enzyme chemically linked to fine carbon particles, and a coating disposed over the enzyme-containing material.

ADVANTAGES OF INVENTION

Similarities and differences between

-the inventive coated-wire needle-type enzyme electrode

-state-of-the-art, including Yacyaych (5,286,364 1994), an ultramicrobiosensor with and without films:

1. The inventive electrode has a Pt base electrode surface. Others use a carbon based electrode surface with a platinized surface - platinium hexachloride particles - a carbon ultramicroelectrode, using carbon fibers. We do not use a mediator. If a mediator is used, then a Pt surface is not needed.

2. The inventive electrode uses fine carbon particles plus Nafion, with the enzyme chemically bound (immobilized, linked to) the surface of the

particles. Thus the inventive electrode has a very large effective surface area. This allows there to be an excess of enzyme in the pool, over that entrapped in the Nafion or the electrodeposited layer. Therefore, the inventive electrode function is independent of the degree of inactivation of the enzyme. As the inventive electrode works in a diffusion-limited mode, the activity of the electrode depends on the properties of the membranes employed, not on the degree of inactivation of the enzyme. Others use free enzyme above the base electrode, and sometimes an electron transfer mediator (such as ferrocene, resorcinol). Construction of their electrodes suggests that their electrodes function in a kinetic regime. As a result of this their electrode activity will be proportional to (or at least depend on) the enzyme activity. Thus the stability and lifetime of their electrodes is far less than of ours. We do not use an electron transfer mediator. Such mediators may be good for very short-term (disposable) sensors, but the mediators are relatively unstable. In any case, such mediators are not suitable for use in humans, being either toxic, or having powerful and often undesirable biochemical interactions.

3. The use of fine carbon particles greatly increases the stability and life of the inventive sensors, compared to others.

4. The inventive electrode operates at an applied voltage (bias) of 0.65V. Others use a different bias, operating their electrode in a different electrochemical regime.

5. The inventive electrode incorporates into the layer on top of the Pt base electrode, a Nafion ionomer to stabilize the sensor and decrease interference. Further, it makes the electrode function in a diffusion-limited mode, and also avoids many interference effects from external body chemicals or chemicals from an industrial process.

6. The inventive electrode has coatings applied on the outside of one or more of PVC, polyurethane, cellulose acetate, silastic. This protects the internal enzymes from external interfering chemicals and fluids.

7. The inventive signal is, compared to others, very large, stable, and noise free.

8. The inventive sensor lifetime is up to 56 days, others achieve at best a few days.

9. The inventive sensor response time is a few seconds to a minute, others are 2 minutes or more.

10. The inventive sensors have a linear range up to 30 mM glucose in blood, others have only a linear range up to about 6-8 mM in buffers, with the use of toxic mediators. Other sensors cannot be used as implantable glucose sensors in humans, as they funtion and respond properly only over the normal to normal-high range of glucose concentration, not into the hyperglycemic range.

11. The inventive sensors responded in blood and

plasma up to 38 mM glucose. Others had responses up to maybe 20 mM before the response flattens, and only with the use of toxic mediators (such as resorcinol, ferrocene etc), undesirable in patients in shock.

12. The design of the inventive sensor is completely different from others.

Description of Preferred Embodiments

Referring now to the drawings in detail, Fig. 1 shows an inventive sensor that was fabricated by entrapping the glucose oxidase enzyme in an electrochemically grown poly(1,3-phenylenediamine) film. This sensor, which is, for example, a needle glucose biosensor, has a working electrode or anode in the form of the platinum wire 1. Except for the end 1', the platinum wire 1 is covered by insulation 2, such as Silastic. Surrounding the insulation 2 is a sensor body 3, which in this embodiment is in the form of a stainless steel needle. Disposed on the exposed bulb end 1' of the platinum wire 1 is enzyme-containing material, in this embodiment in the form of enzyme incorporated in poly(1,3-phenylenediamine) film. A polymer coating 5 is then disposed over the enzyme-containing material 4. Finally, a reference electrode or cathode 6 is connected to the sensor body or needle 3.

In the embodiment illustrated in Fig. 2, a portion of a platinum wire working electrode 1A is again covered with an insulation 2A, such as Silastic, leaving exposed one end of the wire 1. The sensor body or stainless steel needle 3A is disposed on the insulation 2A and in this embodiment extends not only beyond the insulation

but also beyond the exposed end 1'A of the wire 1A. The enzyme-containing material 4A that is disposed over the end 1'A is in this embodiment enzyme incorporated in a platinum black matrix. Disposed over the enzyme-containing material 4A is a coating 5A, for example, Nafion. A reference electrode or cathode 6A is again connected to the needle 3A.

Fig. 3 shows a modified embodiment that can be used in the laboratory and for clinical applications. In this embodiment, the platinum wire 1B has a portion thereof, except for the bulb-like end 1'B, covered by insulation 2B in the form of a heat shrunk tube. Disposed about part of the insulation 2B is a sensor body 3B in the form of an outer wrapping that is preferably made of plastic, although it could also be made of some other material, such as metal. A reference electrode or cathode 6B extends between the insulation 2B and the sensor body 3B and is wrapped around the insulation 2B between the bulb-like end 1'B of the platinum wire and the sensor body 3B. In this embodiment, the enzyme-containing material 4B comprises a conducting or non-conducting polymer. Conducting and nonconducting polymers that can be used include, but are not limited to conducting polymers such as poly(pyrrole), poly(N-methylpyrrole) (P.N. Bartlett and P.G. Whittaker, J. Electroanal. Chem., 224 (1987) 37), poly(aniline) (J.C. Cooper and E.A.H. Hall, Biosensors, 7 (1992) 473); nonconducting polymers such as poly(1,2-phenylenediamine) and poly(1,3-phenylenediamine), poly-(phenol) (J. Wang, S-P Chen and M-S Lin, J. Electroanal. Chem.,

273 (1989) 231; R.L. McClarley, E.A. Irene and R.W. Murray, J. Phys. Chem., 95 (1991) 2492; P.N. Bartlett and D.J. Caruana, Analyst, 117 (1992) 1287. Poly(indole) (P.C. Pandey, J. Chem. Soc. Faraday Trans. I, 84 (1988) 2259) has also been used in the fabrication of glucose biosensors.

Polymer membranes that can be used for the coating 5, as well as the insulation 2, include, for example, polyurethane, polyvinylchloride, Silastic, and cellulose acetate, polytetrafluoroethylene, polycarbonate.

Specific manufacturing examples are as follows:

Sensor Design and Fabrication

The design approach for fabrication of the sensor was chosen to be that of miniaturization to a needle size. For the fulfillment of this requirement a hypodermic needle (approximately 18 gauge -- outer diameter 1.27 mm) was chosen to act as sensor body and as counter and reference electrodes. For the working electrode preparation, three different methods of enzyme immobilization were chosen and investigated, leading to 3 different prototypes.

The fabrication of the sensors involved three principal steps. The first step, which is common to the three sensor designs, involves the initial pretreatment and preparation of the needle body and the platinum electrode. The second step is the immobilization of the glucose oxidase enzyme and its incorporation into the needle body. The third and last step is the formation of the diffusion limiting membrane or coating. The coatings differ according to each immobilization

technique.

The initial preparation and pretreatment of the sensor body and platinum wires were as follows. The stainless steel needle was cut at both ends to remove the plastic cap and the pointed end, and the ends were smoothed using files and fine sand paper. The needle was cleaned in concentrated nitric acid, washed with distilled water and dried. A platinum wire was cleaned in concentrated nitric acid and treated in a propane flame to form a smooth shape or a bulb at one of the ends. The platinum wire was insulated by dipping in Silastic up to 2 mm below the bulb end, or by sealing in a polyethylene tubing with proper inner diameter and was fixed in place by gluing to the sensor body with cyanoacrylate glue. Wires of 0.5 mm diameter were insulated using Teflon heat shrinkable tubing. The partially insulated platinum wire was electrochemically treated by switching between +2.0 V and -1.0 V versus a silver/silver chloride electrode in a three electrode scheme six times for one hour.

Three different sensor designs were investigated, differing in the enzyme immobilization process, and are described below.

First Prototype - Enzyme Entrapped in Non-Conducting Polymer

Figure 1 shows a schematic of a sensor fabricated by entrapping the GOD enzyme in an electrochemically grown poly(1,3-phenylenediamine) film.

The electropolymerization of 1,3-phenylenediamine and the incorporation of the enzyme particles was carried out in a solution containing 3-5 mg of 1,3-phenylenediamine, 20 mg

of Glucose Oxidase and 20 mg of ULTI carbon powder in 9 mL at pH 7.4 and 1 mL of Nafion. This preparation process was performed potentiostatically at +0.65 V versus a silver/silver chloride reference electrode for 15 minutes. An additional coating of poly(1,3-phenylenediamine) was carried out. The formation of this coating was in a solution of 3-5 mg of 1,3-phenylenediamine. This process was performed potentiostatically at +0.65 V versus a silver/silver chloride reference electrode for 10 minutes. The prepared platinum electrode was washed thoroughly in a stirred buffer solution overnight.

A variety of different polymer membranes of varying concentrations were applied to this sensor. The membranes were formed over the sensor tip by dip casting. The sensor end was dipped in the polymer solution for 20 seconds. The sensor was then held vertically and dried in air for one hour. The results of evaluation tests on the above manufactured sensors are given below.

Second Prototype - Enzyme Entrapped in Platinum Black Matrix

Figure 2 shows a schematic of a sensor fabricated by entrapping the GOD enzyme in a platinum black matrix.

The platinization and electrophoretic incorporation of the enzyme particles was carried out in a solution containing 33 mg of sodium hexachloroplatinate and 30 mg of Glucose Oxidase and 0.6 mg of lead acetate in one mL at pH 3.5 (Y. Ikariyama, S. Yamauchi, T. Yukiashi and H. Ushioda, J. Electrochem. Soc., 136 (1989) 702).

This preparation process was performed potentiostatically at -0.2 V versus a silver/silver chloride reference electrode for 20 minutes. The prepared platinum wire was washed thoroughly in a stirred buffer solution overnight. The prepared platinum wire was then inserted into the body of the needle and affixed with epoxy. The platinization procedure was also performed with platinum wire already inserted in the needle body.

Nafion coatings of the sensor were obtained by dipping the sensor end in 0.5% ionomer solution for 30 seconds. The sensor was then held vertically and dried in air for one hour.

Third Prototype - Enzyme Immobilized on Stöber glass

Fig. 3 shows the schematic of the electrode construction. The electrode consists of a platinum wire, a coating layer of Stöber glass beads on the platinum, and the outer immobilized enzyme layer. The glass coating layer has two effects: it acts as a support and matrix for the enzyme; it can be a barrier to some substances that may cause disturbance in the signal.

Silanization was utilized to prepare the Stöber glass beads and the platinum wire surfaces to bond with glutaraldehyde. A solution of 3-aminopropytriethoxy silane (0.2 mL) in distilled water (2 mL, to give a 10% v/v solution) was prepared. The pH was adjusted to 4.1 using hydrochloric acid (HCI) solution. This mixture was subsequently placed into a small vial and several electrode immersed into the vial. They were kept in an oven at 80°C and shaken every

fifteen minutes for three hours. The sensors were rinsed completely in distilled water and then dried at 120°C for three hours. The wires were stored in the refrigerator for 24 hours.

Immobilization was accomplished by first creating a layer of glutaraldehyde bonded to the silanized electrode surface. A 2.5% glutaraldehyde solution was made with 27 mL distilled water and 3 mL of 25% glutaraldehyde. Immobilization was accomplished by immersing the wire in the glutaraldehyde solution, continuously stirred, for one hour at room temperature. The wires were then rinsed with water for one hour at room temperature, after which the electrodes were immersed in the enzyme solution. The enzyme solution was prepared with 80 mg of glucose oxidase in 2 mL phosphate buffer of pH 7.4. The wires were left in the enzyme solution overnight at 4°C to allow immobilization onto the glass layer.

COMPARISON OF DIFFERENT COATINGS FOR FIRST SENSOR PROTOTYPE

Tests with different coatings have been performed, and Table 1 shows a summary of the sensor characteristics obtained with sensors employing five different polymer coatings, namely; cellulose acetate (CA), Silastic 3-5025, Silastic Q7-2213, polyurethane (PU) and polyvinylchloride (PVC). Table 1 presents a variety of concentrations of the polymer coating solutions and the resulting parameters of the calibration curves of the sensors: linearity (as the upper limit of the calibration curve linear range), sensitivity (slope of the linear portion of the calibration

curve) and response time. It should be noted that any coated sensors showing a non-linear response were assumed to have a linear range of 2.2 mM as the linearity below this range was not investigated. It can be seen that extended linearity is not obtained with any of the five polymers below a polymer coating concentration of 5%. At a concentration of 5% of PVC and CA solutions and 7% of PU solutions a linearity of up to 8.9, 11.1 and 13.3 mM respectively was obtained. It is also noted that the use of the Silastic coatings even at very high polymer solution concentrations did not show a significant increase in the linearity, hence the use of Silastic as an outer polymer membrane was abandoned. Increasing the polymer coating solution concentrations extends the linear range of the sensor response further, but is accompanied by a corresponding decrease in the sensor sensitivity. This can be explained by assuming that increasing the polymer coating concentration produces a thicker or less porous coating. Increasing the coating thickness or decreasing its porosity limits the flux of glucose and thus decreases the amount of hydrogen peroxide produced, reducing the response to a given glucose concentration.

Table 1. Polymer coatings and their effect on the linearity, sensitivity and response time of the prepared first prototype sensors.

Coating	Concentration (mg %)	Linearity (mM)	Sensitivity (nA/mM)	Response time (secs)
Cellulose Acetate	0.2	2.2	10.8	69
	1	2.2	5.4	69
	5	11.1	3.15	120
	7.5	17.7	2.25	120
	10	26.7	1.62	183
	15	No response		
Silastic 3-5025	10	2.2	21.4	55
	20	2.2	16.7	58
	30	2.2	9.4	58
	33	6.7	4.05	60
Silastic Q7-2213	60	6.7	24.3	120
	80	15.6	4.95	120
Polyurethane	0.5	2.2	34.74	19
	1	2.2	18.9	19
	5	2.2	13.95	22
	7	13.3	4.14	22
	10	17.8	2.16	24
	12	31.1	1.35	24
	15	No response		
Polyvinylchloride	0.2	2.2	23.76	32
	1	2.2	17.57	32
	5	8.9	12.15	34
	8	17.7	2.7	35
	10	37.8	1.8	35
	12	No response		

USE OF ANTICOAGULANT

In thromboembolic conditions it is well known that it is desirable to delay the coagulation process to a certain degree. Therefore, various anticoagulants have been developed for treatment of these conditions. The ones most useful clinically are heparin and

Dicumarol.

PLASMA TESTS

In vitro plasma tests try to simulate the working environment in the body. It can be seen from the previous discussion that all three sensor prototypes responded well in plasma showing close correlation with their performance in buffer. Some decrease of sensitivity may be observed in the case of the second and third sensor prototypes. This is expected due to adsorption of proteins on the sensor surface. No decrease in sensitivity is
observed for the first sensor prototype.

Table 2 shows the evaluation of the second prototype sensor performance in plasma and in buffer solution before and after the plasma test. Comparing the sensor response in buffer solution before the plasma test with that during the plasma test, it can be seen that the values of the sensor response to glucose concentration from 10 to 12 mM in both measurements coincide (within 2%). At higher glucose concentration the signal in plasma is lower than that obtained in buffer solution but with an acceptable deviation of 7%. A higher response of the sensor to glucose levels in plasma within the range 4.4 - 8.4 mM is observed. The values of the sensor response

obtained in buffer after the plasma test practically coincide with those obtained in plasma.

Table 2. Second sensor prototype response to glucose in buffer solution and in bovine plasma.

Glucose Concentration mM	Sensor Response in Buffer Solution Before Plasma Test nA	Bovine Plasma nA	Buffer Solution After Plasma Test nA
4.4	63	92	104
6.6	94	122	127
158.4	137	157	155
10.2	186	187	188
12.1	201	205	205
15.6	230	213	223

INTERFERENCE TESTS

The effect of physiological interferents on the sensor signal is also another important aspect to be considered as the sensor will be implanted in the body. The effect of interferents was tested by five common substances. The first sensor prototype shows a decrease in sensitivity after the addition of interferents. The second sensor prototype shows a decreased effect of glycine, urea and ascorbic acid. There is a marked effect of uric acid on the sensor signal as well as a decrease in sensor sensitivity. The second sensor prototype completely eliminates the effects of glycine and urea while greatly reducing the effect of uric and ascorbic acid. There is also no decrease in sensor

sensitivity as a result of adding the interferents. Similar results were obtained for the third prototype.

Interference tests have been performed, and Table 3 shows the effect of addition of carbohydrates and other common interferents. It can be seen that the response to carbohydrates (other than glucose) is negligible with respect to the sensor response to the same concentration of glucose. This indicates that the sensor signal is due to the oxidation of glucose by GOD and not the electrochemical oxidation of glucose on the platinum electrode surface. Nafion coatings caused a 50% reduction in the response of the sensor to other common interferents.

Table 3. Second sensor prototype response of non-coated and Nafion coated sensors to carbohydrates and other common interferents.

Substance	Concentration	Sensor response	
	mM	Non-coated nA	Nafion coated nA
Glucose	4.4	465	105
Sucrose	4.4	8	< 1
Fructose	4.4	6	< 1
Lactose	4.4	2	< 1
Glycine	10	35	21
Urea	5	25	17
Ascorbic Acid	6	28	14
Acetaminophen	20	420	172
Uric Acid	15	981	352

OVERALL PERFORMANCE OF PROTOTYPE SENSORS

The overall performances of the three manufactured sensor prototypes are compared in Table 4. The three sensor prototypes are based on the immobilization of glucose oxidase in a poly(1,3-phenylenediamine) film (first prototype), in a platinum black matrix (second prototype), or on Stöber glass bead (third prototype). The ultimate goal of the sensor development will be its use in intravascular glucose monitoring and especially in victims in risk of hemorrhagic shock. A further use is in monitoring glucose levels in a chemical process. Thus, a comparison of the performance of each of the three sensor prototypes and how well they satisfy the constraints set forth by these goals would be helpful in evaluating which sensor

prototype will be used.

Hemorrhagic shock is characterized by the alteration of glucose concentrations between the hypoglycemic and hyperglycemic levels. To simplify calibration and operation of the sensor, the sensor response should be linear in glucose concentration over the range of interest. This linearity should be at least 22 mM (400 mg/dL) to assure linear output of the sensor within some safety margin. It can be seen from Table 4 that all sensor prototypes satisfy this requirement. The linearity of the sensor output signal versus the glucose concentration is extended to at least 22 mM. The first sensor prototype showed a linearity of 26.7 mM with sensitivity of 1.62 nA/mM, 31.1 mM with a sensitivity of 1.35 nA/mM, and 37.7 mM with a sensitivity of 1.8 nA/mM for cellulose acetate (CA), polyurethane (PU) and polyvinylchloride (PVC) coated sensors, respectively. The second sensor prototype (with enzyme entrapped in a platinum black matrix) showed a linearity of up to 33 mM. The third sensor prototype also showed a linearity of at least 22 mM.

Another requirement for monitoring glucose levels during hemorrhagic shock is a fast sensor response (short response time) to changes

in glucose concentration. Comparing the response times of the three sensor prototypes, the first sensor prototype shows response times of 183, 24 and 35 seconds for CA, PU and PVC coated sensors, while the second sensor prototype shows a response time of 60 seconds; the third prototype shows a resonse time of 90 seconds. Another test reflecting the response times of the sensors is the in vitro hemorrhagic shock simulation test. This test reflects the response times of the sensors while responding to large changes in glucose concentration (between hypoglycemic and hyper glycemic levels). Table 4 shows the first sensor prototype having an outer PVC membrane shows a response time of 1.7 minutes for the increasing and 5.1 minutes for the decreasing step. The second sensor prototype shows a response time of 5 minutes for the increasing and 10 minutes for the decreasing step changes. It is thus seen that the first and second sensor prototypes show the shortest response times in response to both small and large changes in glucose concentration.

Table 4. A comparison of the performance of the three developed sensor prototypes with the different external polymer membranes.

Performance	First Prototype	Second Prototype	Third Prototype
Linearity	CA: 26.7		

(mM)	PU: 31.1 PVC: 37.7	33	PVC: 22
Sensitivity (nA/mM)	CA: 1.62 PU: 1.35 PVC: 1.80	36	PVC: 0.82
Response Time (seconds)	CA: 183 PU: 24 PVC: 35	60	PVC: 90
Hemorrhagic Shock: Duration of Increasing Glucose Step (minutes)	PVC: 1.7	5	
Duration of Decreasing Glucose Step (minutes)	PVC: 1.5	10	
Signal Variation	< 5%	< 10%	
Effect of Interferents	No change in Sensitivity	Decreased Sensitivity	
Plasma Test	Decreased sensitivity	Decreased Sensitivity	No change in Sensitivity

PC: polycarbonate CA: cellulose acetate PU: polyurethane
N PC: Nafion coated polycarbonate S PC: silastic coated polycarbonate

The reproducibility of the sensor signal is good in all three sensor prototypes. The variations in the sensor response do not exceed 5% in the first prototype response, while it is less than 10% for the second prototype.

All three prototypes showed a life time of at least one week with no significant change in the sensor performance, hence satisfying the life time requirement.

One last aspect to be considered is the ease of fabrication of the sensor prototypes. In general, the three sensor prototypes are easily fabricated. The first prototype has the advantage of electrochemical growth of the entrapping film, hence allowing better controllability on the thickness of the film and hence the amount of enzyme entrapped.

The present invention is, of course, in no way restricted to the specific disclosure of the specification, Examples and drawings, but also encompasses any modifications within the scope of the appended claims.

CLAIMS:

1. A coated wire sensor, characterized by:

a platinum wire (1) as a working electrode, said wire having a first end (1');

a sensor body (3) in the form of a hollow member disposed about at least a portion of said platinum wire (1), said sensor body (3) being spaced from said platinum wire;

a reference electrode (6) that is operatively associated with said first end (1') of said platinum wire (1) but is not in contact with said wire;

insulation (2) disposed along at least a portion of a length of said platinum wire (1) between said wire and at least one of said sensor body (3) and said reference electrode (6);

enzyme-containing material (4) disposed on said first end (1') of said platinum wire (1), said enzyme-containing material (4) comprising enzyme chemically linked to fine carbon particles; and

a coating (5) disposed over said enzyme-containing material (4).

2. A sensor according to claim 1, wherein said sensor body (3, 3B) is in the form of a hollow stainless steel member or a hollow

plastic member which can have a diameter of, for example, approximately 1 mm.

3. A sensor according to claim 2, wherein said hollow stainless steel member (3) extends to the vicinity of said first end (1') of said platinum wire (1), and said reference electrode (6) is connected to said hollow stainless steel member (3) on a side thereof remote from said insulation (2).

4. A sensor according to claim 2, wherein said reference electrode (6B) is disposed about a portion of said insulation (2B) in the vicinity of said first end (1'B) of said platinum wire (1B).

5. A sensor according to claim 1, wherein said insulation (2) is selected from the group consisting of polyurethane, polyvinylchloride, polyacrylate, Silastic, polytetrafluoroethylene, polycarbonate, and cellulose acetate, and said enzyme-containing material (4) is selected from the group consisting of enzyme incorporated in a platinum black matrix, enzyme incorporated in poly (1,3-phenylenediamine) film, enzyme incorporated in poly (1,2-phenylenediamine), enzyme incorporated in 1,2 diaminobenzene and enzyme incorporated in 1,3 diaminobenzene, and said coating (5) is a polymer

selected from the group consisting of polyurethane, polyvinylchloride, Silastic, and cellulose acetate.

6. A sensor according to claim 5, wherein an anti-clotting agent is added to said coating (5) such as heparin.

7. A sensor according to claim 1, wherein said enzyme chemically linked to fine carbon particles is in a stabilizing agent.

8. A sensor according to claim 7, wherein said stabilizing agent is selected from the group consisting of Nafion, and polyvinylalcohol.

9. A sensor according to claim 1, wherein said first end (1', 1'A) of said platinum wire (1, 1A), with said enzyme-containing material (4, 4A) and said coating (5, 5A) thereof, has a rounded or squared-off configuration.

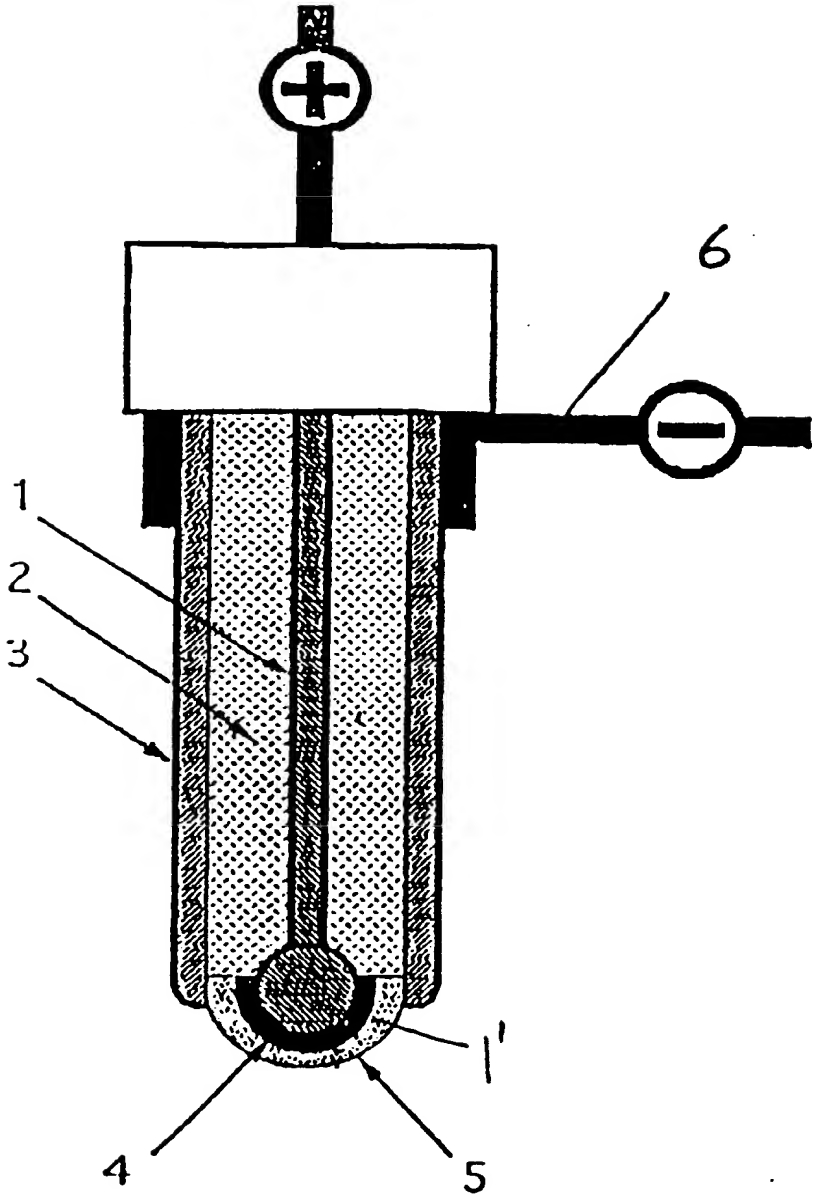

FIG 1

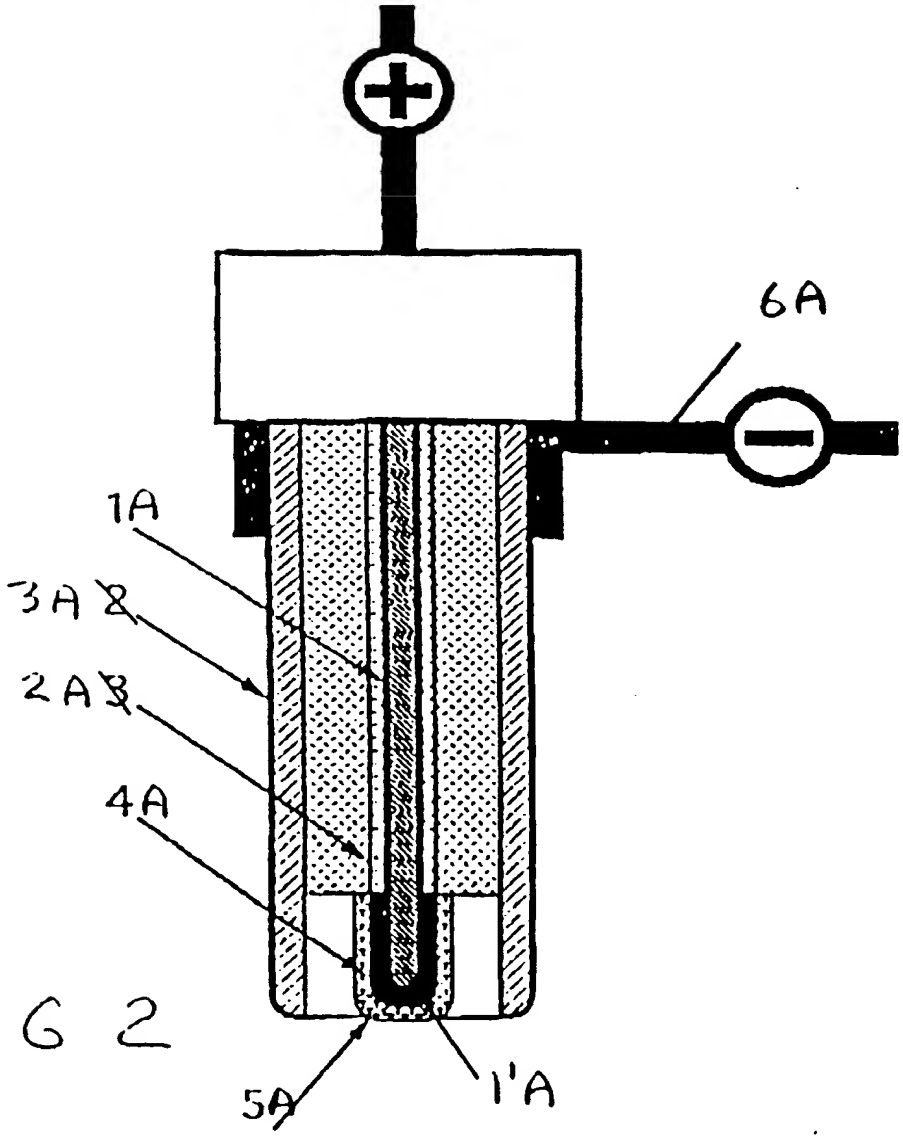

FIG 2

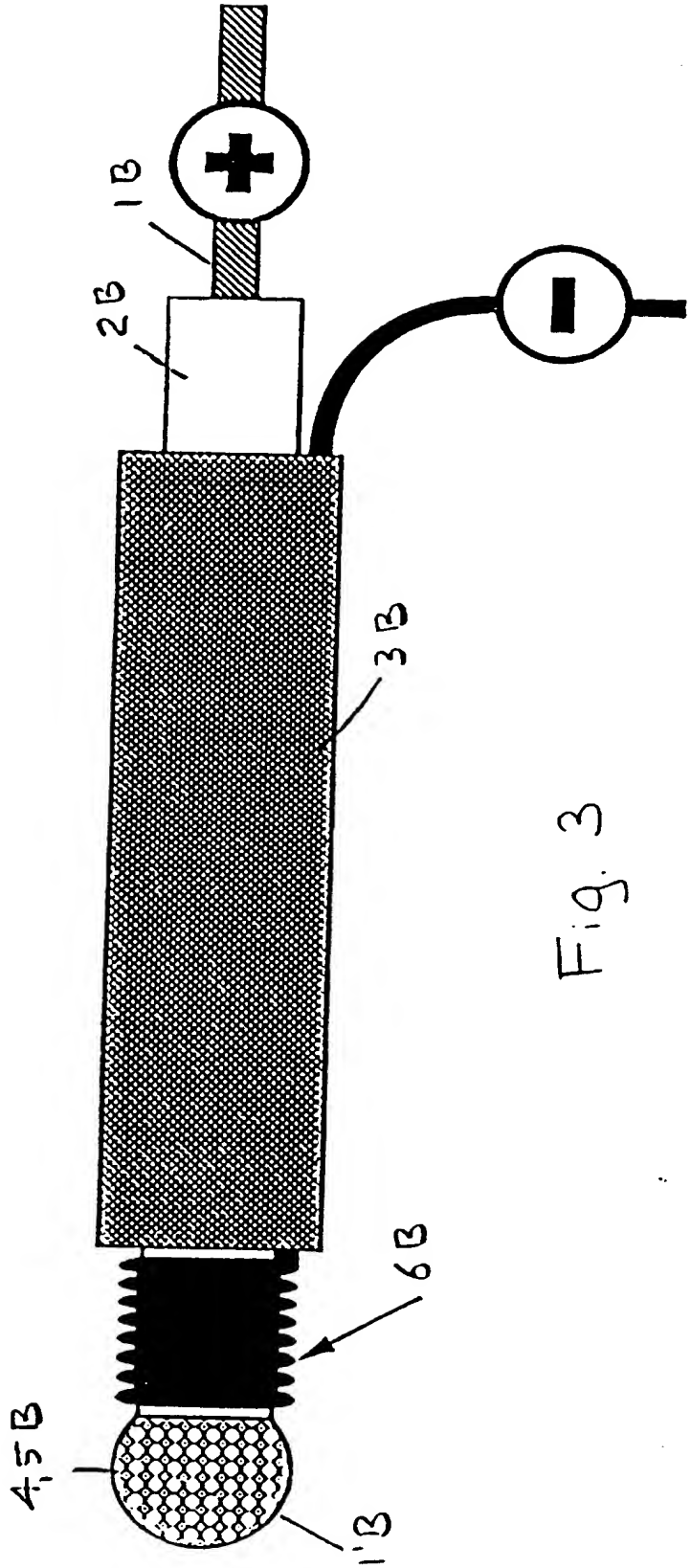

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17893

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 27/327
US CL :204/402
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/402, 415; 205/777.5, 778, 792

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,919,141 (ZIER ET AL) 24 April 1990, see column 4, line 54 to column 5, line 13 and column 7, line 24.	1-9
Y	US, A, 5,089,112 (SKOTHEIM ET AL) 18 February 1992, see column 5, line 34 to column 6, line 65.	1-9
Y	US, A, 5,272,087 (EL MURR ET AL), 21 December 1993, see column 2, lines 56-58 and column 5, line 40.	2-4
Y	SASSO, S.V. Electropolymerized 1,2-Diaminobenzene as a Means to Prevent Interferences and Fouling and to Stabilize Immobilized Enzyme in Electrochemical Biosensor. Anal, Chem. 1990, Vol. 62, pages 1111-1117, see the abstract.	5-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17893

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GEISE, R.J. Electropolymerized 1,3-Diaminobezene for the construction of a 1,1' - Dimethylferrocene Mediated Glucose Biosensor. Anal. Chim. Acta. 1993, Vol. 281, pages 467-471, see page 470 left column.	5-6
Y	IKARIYAMA, Y. Electrochemical Fabrication of Amperometric Microenzyme Sensor. J. Electrochem. Soc. March, 1989, Vol. 136, pages 702-706.	5-6

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(71)(72) Applicant and Inventor: WILKINS, Ebtisam, S. [US/US]; 9961 Florence Avenue N.E., Albuquerque, NM 87122 (US).

(74) Agents: BECKER, Robert, W. et al.; Suite B, 11896 N. Highway 14, Tijeras, NM 87059 (US).

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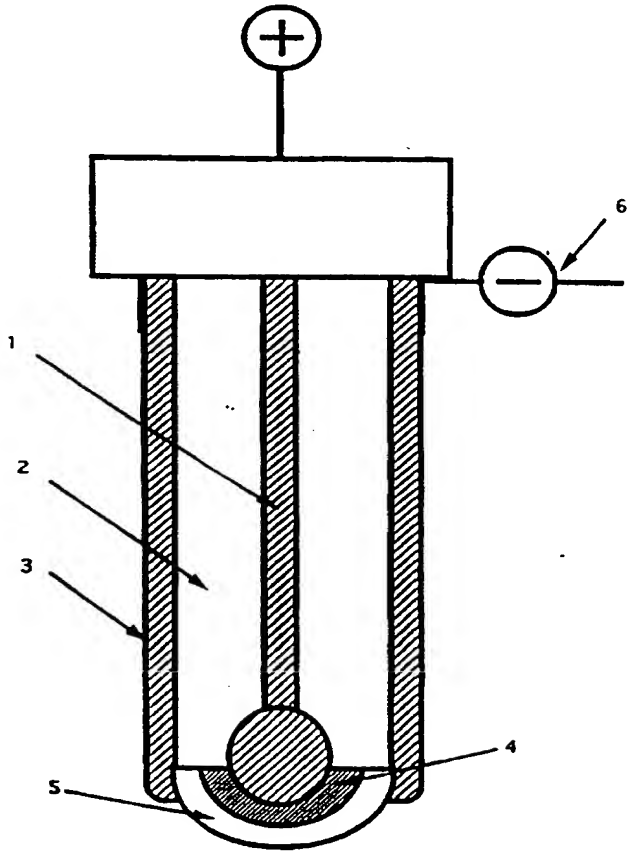
(54) Title: COATED WIRE SENSOR

(57) Abstract

A coated wire sensor, including a platinum wire (1) as a working electrode, a sensor body (3) in the form of a hollow member disposed about at least a portion of the platinum wire, the sensor body being spaced from the platinum wire, a reference electrode (6) that is operatively associated with a first end of the platinum wire, but is not in contact with the wire, insulation (2) disposed along at least a portion of a length of the platinum wire between the wire and the sensor body and/or the reference electrode, enzyme-containing material (4) disposed on the first end of the platinum wirer, the enzyme-containing material comprising enzyme chemically linked to fine carbon particles, and a coating (5) disposed over the enzyme-containing material.



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COATED WIRE SENSOR

Technical Field

The present invention relates to a coated wire sensor, which can be used in the laboratory, has clinical applications, has applications in the food and pharmaceutical industries, and in particular can be used in the management of hemorrhagic shock.

Among other uses, a coated wire sensor can be used to increase the survival rate of injured patients who are at risk of hemorrhagic shock, especially while they are being transported to a hospital. The important point in such an application is that unlike diabetes, hemorrhagic shock requires direct intravascular implantation of such sensors, and the monitoring of glucose levels for up to several hours.

For example, hemorrhagic shock is characterized by the alteration of the glucose concentrations between the hypoglycemic and hyperglycemic levels. Hence, a linearity of the sensor signal versus the glucose concentration is required. This linearity should be at least 22 mM (400 mg/dL) to assure linear output of the sensor within a safety margin. Unfortunately, the heretofore known devices do not provide for a high enough linearity.

It is therefore an object of the present invention to provide a coated wire sensor that has a linear range of at least 22 mM, as well as a short response time and as small an effect of common physiological and industrial fermentation process interference on the sensor signal as possible. Furthermore, the sensor should also have a lifetime of at least five days with no

significant change in the sensor signal during this time period.

Background Art

Enzyme Entrapped in Conducting Polymers

Electropolymerization involves the electrochemical oxidation of a monomer from a solution containing the enzyme to form a conducting or non-conducting polymer on the electrode surface. This approach has several advantages. First, this process can be governed by the electrode potential, and therefore allows accurate control of the polymer film thickness and hence the amount of entrapped enzyme. Second, since the polymerization occurs locally at the electrode surface, it can be used to confine an enzyme precisely at the electrode without cross-immobilizing it on a neighboring electrode. This property is suitable for the fabrication of micro-arrays. Third, it is possible to use this technique for building multilayer structures, either one or more enzymes layered within a single polymer, or one enzyme within a multilayered copolymer.

Foulds and Lowe (N.C. Foulds and C.R. Lowe, J. Chem. Soc., Faraday Trans., 82 (1986) 1259) and Umana and Waller (M. Umana and J. Waller, Anal. Chem., 58 (1986) 2979) were the first to demonstrate the possibility of employing GOD (glucose oxidose) entrapped in poly(pyrrole) in the fabrication of amperometric glucose sensors. Since then, a large amount of research effort has been directed towards the development of glucose sensors employing this concept (P.N. Bartlett and J.M. Cooper, J. Electroanal. Chem., 362 (1993) 1). Fortier et al. investigated and

reported on the concentrations of pyrrole and GOD which led to optimum sensor response (G. Fortier, E. Brassard and D. Belanger, Biosensors and Biolectronics, 5 (1990) 473). They reported that these optimum conditions led to a biosensor with a high enzyme loading and a longer lifetime.

There have been other approaches to the fabrication of pyrrole sensors besides physical entrapment of GOD. Covalent electropolymerization of GOD to pyrrole by modifying different side chains on the enzyme has been reported (S.E. Wolawacz, B.F. Yon-Hin and C.R. Lowe, Anal. Chem., 64 (1992) 1541). The modification reactions involved carbodiimide coupling or Schiff base formation (B.F. Yon-Hin, M. Smolander, T. Crompton and C.R. Lowe, Anal. Chem., 65 (1993) 2067). Foulds et al also demonstrated the possible use of covalently attached ferrocenes to pyrrole monomers as a step towards the fabrication of reagentless sensors (N.C. Foulds and C.R. Lowe, Anal. Chem., 60 (1988) 2473). There have also been other reports on incorporating mediators in pyrrole films as anionic counter ions (Y. Kajiya, H. Sugai, C. Iwakura and H. Yoneyama, Anal. Chem., 63 (1991 49; S. Yabuki, H. Shinohara, Y. Ikariyama and M. Aizawa, J. Electroanal. Chem. 277 (1990) 179).

Although most of the research has focused on poly(pyrrole) sensors, there have been reports of using other conducting polymers such as poly(N-methylpyrrole) (P.N. Bartlett and P.G. Whittaker, J. Electroanal. Chem., 224 (1987) 37), poly(aniline) J.C. Cooper and E.A.H. Hall, Biosensors, 7 (1992) 473).

Enzyme Entrapped in Non-Conducting Polymers

Non-conducting polymers have been studied as they have several advantageous characteristics not found in the conducting polymers. First, their growth is self limiting, thus producing thin polymer films. This results in a short sensor response time. The thin film also allows a higher enzyme content leading to a large sensor response and a high sensitivity. Non-conducting polymers also have a characteristic permselectivity, greatly decreasing the effect of common physiological interferents on the sensor response.

A non-conducting polymer that has been the focus of much research is 1,2-phenylenediamine.

Yacynych et al. were the first to demonstrate that the oxidation of 1,2-phenylenediamine is irreversible and formed an insulating polymer film completely covering the electrode surface (A.M. Yacynych and H.B. Mark, J. Electrochem. Soc., 123 (1976) 1346). Heineman et al demonstrated that 1,2-phenylenediamine forms a polymeric film over the pH range from 4 to 10 (W.R. Heineman, H.J. Wick, and A.M. Yacynych, Anal. Chem., 52 (1980) 345). After the possibility of entrapping GOD in these non-conducting polymer films had been demonstrated, numerous studies have appeared applying this concept in the fabrication of glucose sensors.

Sasso et al. proposed a glucose sensor having a platinized reticulated vitreous carbon electrode with GOD entrapped in a poly(1,2-phenylenediamine) film (S.V. Sasso, R.J. Pierce, R. Walla, and A.M.

Yacynych, Anal. Chem., 62 (1990) 1111). Their sensor was used in Flow Injection Analysis for determining glucose levels in human serum. They demonstrated the ability of the poly(1,2-phenylenediamine) film in increasing the thermal stability of the GOD enzyme. Malitesta et al. constructed a glucose sensor, employing the same principle, but using platinum electrodes (C. Malitesta, F. Palmisano, L. Torsi and P.G. Zambonin, Anal. Chem., 62 (1990) 2735). Their sensor had a response time of 1 second and demonstrated almost complete rejection of common physiological interferents, especially ascorbate. Wang et al. used a similar construction but with an additional outer lipid layer (J. Wang and H. Wu, Anal. Chim. Acta, 283 (1993) 683). Their sensor showed a response time of 8 to 10 seconds. They demonstrated that the extra lipid layer almost completely eliminated the sensor response to acetaminophen (another common physiological interferent).

Another non-conducting polymer that has been studied is poly(1,3-phenylenediamine). This polymer is not commonly used in the construction of glucose sensors although it possesses the same permselective properties as poly(1,2-phenylenediamine). Geise et al. have demonstrated the use of 1,1'-dimethylferrocene as a mediator entrapped with GOD in a poly(1,3-phenylenediamine) film (R.J. Geise, S.Y. Roach and A.M. Yacynych, Anal. Chim. Acta, 281 (1993) 467). They report that this combination results in a sensor with a linear range of more than 22 mM and a lifetime of four months. Yacynych et al. have also used poly(1,3-phenylenediamine)

for the fabrication of miniaturized glucose sensors (A.M. Yacynych, and E.R. Reynolds, Third World Congress: Biosensors '94, 1-3 June 1994, Proceedings). They report their sensors to have a response time of 40 seconds and decreased fouling at the sensor surface (see also Yacynych et al, U.S. Patent 5,286,364).

There are reports on the use of other non-conducting polymers, such as poly-(phenol) (J. Wang, S-P Chen and M-S Lin, J. Electroanal. Chem., 273 (1989) 231; R.L. McClarley, E.A. Irene and R.W. Murray, J. Phys. Chem., 95 (1991) 2492; P.N. Bartlett and D.J. Caruana, Analyst, 117 (1992) 1287). Poly(phenol) has not been used as extensively as poly(phenylenediamine), probably because of its oxidative properties if it comes into contact with air and oxygen and its light sensitivity. Poly(indole) [53] has also been used in the fabrication of glucose biosensors.

Brief Description of the Drawings

These and other objects and advantages of the present invention will appear more clearly from the following specification in conjunction with the accompanying schematic drawings, in which:

Fig. 1 is a cross-sectional view of a first exemplary embodiment of the inventive coated wire sensor;

Fig. 2 is a cross-sectional view of a second exemplary embodiment of an inventive coated wire sensor; and

Fig. 3 shows a third exemplary embodiment of an inventive coated wire sensor.

Disclosure of the Invention

The coated wire sensor of the present

invention is characterized primarily by: a platinum wire as a working electrode, a sensor body in the form of a hollow member disposed about at least a portion of the platinum wire, with the sensor body being spaced from the wire, a reference electrode that is operatively associated with a first end of the platinum wire but is not in contact with the wire, insulation disposed along at least a portion of the length of the platinum wire between the wire and the sensor body and/or the reference electrode, enzyme-containing material disposed on the first end of the platinum wire, with this enzyme-containing material comprising enzyme chemically linked to fine carbon particles, and a coating disposed over the enzyme-containing material.

ADVANTAGES OF INVENTION

Similarities and differences between

-the inventive coated-wire needle-type enzyme electrode

-state-of-the-art, including Yacyaych (5,286,364 1994), an ultramicrobiosensor with and without films:

1. The inventive electrode has a Pt base electrode surface. Others use a carbon based electrode surface with a platinized surface - platinium hexachloride particles - a carbon ultramicroelectrode, using carbon fibers. We do not use a mediator. If a mediator is used, then a Pt surface is not needed.

2. The inventive electrode uses fine carbon particles plus Nafion, with the enzyme chemically bound (immobilized, linked to) the surface of the

particles. Thus the inventive electrode has a very large effective surface area. This allows there to be an excess of enzyme in the pool, over that entrapped in the Nafion or the electrodeposited layer. Therefore, the inventive electrode function is independent of the degree of inactivation of the enzyme. As the inventive electrode works in a diffusion-limited mode, the activity of the electrode depends on the properties of the membranes employed, not on the degree of inactivation of the enzyme. Others use free enzyme above the base electrode, and sometimes an electron transfer mediator (such as ferrocene, resorcinol). Construction of their electrodes suggests that their electrodes function in a kinetic regime. As a result of this their electrode activity will be proportional to (or at least depend on) the enzyme activity. Thus the stability and lifetime of their electrodes is far less than of ours. We do not use an electron transfer mediator. Such mediators may be good for very short-term (disposable) sensors, but the mediators are relatively unstable. In any case, such mediators are not suitable for use in humans, being either toxic, or having powerful and often undesirable biochemical interactions.

3. The use of fine carbon particles greatly increases the stability and life of the inventive sensors, compared to others.

4. The inventive electrode operates at an applied voltage (bias) of 0.65V. Others use a different bias, operating their electrode in a different electrochemical regime.

5. The inventive electrode incorporates into the layer on top of the Pt base electrode, a Nafion ionomer to stabilize the sensor and decrease interference. Further, it makes the electrode function in a diffusion-limited mode, and also avoids many interference effects from external body chemicals or chemicals from an industrial process.

6. The inventive electrode has coatings applied on the outside of one or more of PVC, polyurethane, cellulose acetate, silastic. This protects the internal enzymes from external interfering chemicals and fluids.

7. The inventive signal is, compared to others, very large, stable, and noise free.

8. The inventive sensor lifetime is up to 56 days, others achieve at best a few days.

9. The inventive sensor response time is a few seconds to a minute, others are 2 minutes or more.

10. The inventive sensors have a linear range up to 30 mM glucose in blood, others have only a linear range up to about 6-8 mM in buffers, with the use of toxic mediators. Other sensors cannot be used as implantable glucose sensors in humans, as they funtion and respond properly only over the normal to normal-high range of glucose concentration, not into the hyperglycemic range.

11. The inventive sensors responded in blood and

plasma up to 38 mM glucose. Others had responses up to maybe 20 mM before the response flattens, and only with the use of toxic mediators (such as resorcinol, ferrocene etc), undesirable in patients in shock.

12. The design of the inventive sensor is completely different from others.

Description of Preferred Embodiments

Referring now to the drawings in detail, Fig. 1 shows an inventive sensor that was fabricated by entrapping the glucose oxidase enzyme in an electrochemically grown poly(1,3-phenylenediamine) film. This sensor, which is, for example, a needle glucose biosensor, has a working electrode or anode in the form of the platinum wire 1. Except for the end 1', the platinum wire 1 is covered by insulation 2, such as Silastic. Surrounding the insulation 2 is a sensor body 3, which in this embodiment is in the form of a stainless steel needle. Disposed on the exposed bulb end 1' of the platinum wire 1 is enzyme-containing material, in this embodiment in the form of enzyme incorporated in poly(1,3-phenylenediamine) film. A polymer coating 5 is then disposed over the enzyme-containing material 4. Finally, a reference electrode or cathode 6 is connected to the sensor body or needle 3.

In the embodiment illustrated in Fig. 2, a portion of a platinum wire working electrode 1A is again covered with an insulation 2A, such as Silastic, leaving exposed one end of the wire 1. The sensor body or stainless steel needle 3A is disposed on the insulation 2A and in this embodiment extends not only beyond the insulation

but also beyond the exposed end 1'A of the wire 1A. The enzyme-containing material 4A that is disposed over the end 1'A is in this embodiment enzyme incorporated in a platinum black matrix. Disposed over the enzyme-containing material 4A is a coating 5A, for example, Nafion. A reference electrode or cathode 6A is again connected to the needle 3A.

Fig. 3 shows a modified embodiment that can be used in the laboratory and for clinical applications. In this embodiment, the platinum wire 1B has a portion thereof, except for the bulb-like end 1'B, covered by insulation 2B in the form of a heat shrunk tube. Disposed about part of the insulation 2B is a sensor body 3B in the form of an outer wrapping that is preferably made of plastic, although it could also be made of some other material, such as metal. A reference electrode or cathode 6B extends between the insulation 2B and the sensor body 3B and is wrapped around the insulation 2B between the bulb-like end 1'B of the platinum wire and the sensor body 3B. In this embodiment, the enzyme-containing material 4B comprises a conducting or non-conducting polymer. Conducting and nonconducting polymers that can be used include, but are not limited to conducting polymers such as poly(pyrrole), poly(N-methylpyrrole) (P.N. Bartlett and P.G. Whittaker, J. Electroanal. Chem., 224 (1987) 37), poly(aniline) (J.C. Cooper and E.A.H. Hall, Biosensors, 7 (1992) 473); nonconducting polymers such as poly(1,2-phenylenediamine) and poly(1,3-phenylenediamine), poly-(phenol) (J. Wang, S-P Chen and M-S Lin, J. Electroanal. Chem.,

273 (1989) 231; R.L. McClarley, E.A. Irene and R.W. Murray, J. Phys. Chem., 95 (1991) 2492; P.N. Bartlett and D.J. Caruana, Analyst, 117 (1992) 1287. Poly(indole) (P.C. Pandey, J. Chem. Soc. Faraday Trans. I, 84 (1988) 2259) has also been used in the fabrication of glucose biosensors.

Polymer membranes that can be used for the coating 5, as well as the insulation 2, include, for example, polyurethane, polyvinylchloride, Silastic, and cellulose acetate, polytetrafluoroethylene, polycarbonate.

Specific manufacturing examples are as follows:

Sensor Design and Fabrication

The design approach for fabrication of the sensor was chosen to be that of miniaturization to a needle size. For the fulfillment of this requirement a hypodermic needle (approximately 18 gauge -- outer diameter 1.27 mm) was chosen to act as sensor body and as counter and reference electrodes. For the working electrode preparation, three different methods of enzyme immobilization were chosen and investigated, leading to 3 different prototypes.

The fabrication of the sensors involved three principal steps. The first step, which is common to the three sensor designs, involves the initial pretreatment and preparation of the needle body and the platinum electrode. The second step is the immobilization of the glucose oxidase enzyme and its incorporation into the needle body. The third and last step is the formation of the diffusion limiting membrane or coating. The coatings differ according to each immobilization

technique.

The initial preparation and pretreatment of the sensor body and platinum wires were as follows. The stainless steel needle was cut at both ends to remove the plastic cap and the pointed end, and the ends were smoothed using files and fine sand paper. The needle was cleaned in concentrated nitric acid, washed with distilled water and dried. A platinum wire was cleaned in concentrated nitric acid and treated in a propane flame to form a smooth shape or a bulb at one of the ends. The platinum wire was insulated by dipping in Silastic up to 2 mm below the bulb end, or by sealing in a polyethylene tubing with proper inner diameter and was fixed in place by gluing to the sensor body with cyanoacrylate glue. Wires of 0.5 mm diameter were insulated using Teflon heat shrinkable tubing. The partially insulated platinum wire was electrochemically treated by switching between +2.0 V and -1.0 V versus a silver/silver chloride electrode in a three electrode scheme six times for one hour.

Three different sensor designs were investigated, differing in the enzyme immobilization process, and are described below.

First Prototype - Enzyme Entrapped in Non-Conducting Polymer

Figure 1 shows a schematic of a sensor fabricated by entrapping the GOD enzyme in an electrochemically grown poly(1,3-phenylenediamine) film.

The electropolymerization of 1,3-phenylenediamine and the incorporation of the enzyme particles was carried out in a solution containing 3-5 mg of 1,3-phenylenediamine, 20 mg

of Glucose Oxidase and 20 mg of ULTI carbon powder in 9 mL at pH 7.4 and 1 mL of Nafion. This preparation process was performed potentiostatically at +0.65 V versus a silver/silver chloride reference electrode for 15 minutes. An additional coating of poly(1,3-phenylenediamine) was carried out. The formation of this coating was in a solution of 3-5 mg of 1,3-phenylenediamine. This process was performed potentiostatically at +0.65 V versus a silver/silver chloride reference electrode for 10 minutes. The prepared platinum electrode was washed thoroughly in a stirred buffer solution overnight.

A variety of different polymer membranes of varying concentrations were applied to this sensor. The membranes were formed over the sensor tip by dip casting. The sensor end was dipped in the polymer solution for 20 seconds. The sensor was then held vertically and dried in air for one hour. The results of evaluation tests on the above manufactured sensors are given below.

Second Prototype - Enzyme Entrapped in Platinum Black Matrix

Figure 2 shows a schematic of a sensor fabricated by entrapping the GOD enzyme in a platinum black matrix.

The platinization and electrophoretic incorporation of the enzyme particles was carried out in a solution containing 33 mg of sodium hexachloroplatinate and 30 mg of Glucose Oxidase and 0.6 mg of lead acetate in one mL at pH 3.5 (Y. Ikariyama, S. Yamauchi, T. Yukiashi and H. Ushioda, J. Electrochem. Soc., 136 (1989) 702).

This preparation process was performed potentiostatically at -0.2 V versus a silver/silver chloride reference electrode for 20 minutes. The prepared platinum wire was washed thoroughly in a stirred buffer solution overnight. The prepared platinum wire was then inserted into the body of the needle and affixed with epoxy. The platinization procedure was also performed with platinum wire already inserted in the needle body.

Nafion coatings of the sensor were obtained by dipping the sensor end in 0.5% ionomer solution for 30 seconds. The sensor was then held vertically and dried in air for one hour.

Third Prototype - Enzyme Immobilized on Stöber glass

Fig. 3 shows the schematic of the electrode construction. The electrode consists of a platinum wire, a coating layer of Stöber glass beads on the platinum, and the outer immobilized enzyme layer. The glass coating layer has two effects: it acts as a support and matrix for the enzyme; it can be a barrier to some substances that may cause disturbance in the signal.

Silanization was utilized to prepare the Stöber glass beads and the platinum wire surfaces to bond with glutaraldehyde. A solution of 3-aminopropytriethoxy silane (0.2 mL) in distilled water (2 mL, to give a 10% v/v solution) was prepared. The pH was adjusted to 4.1 using hydrochloric acid (HCI) solution. This mixture was subsequently placed into a small vial and several electrode immersed into the vial. They were kept in an oven at 80°C and shaken every

fifteen minutes for three hours. The sensors were rinsed completely in distilled water and then dried at 120°C for three hours. The wires were stored in the refrigerator for 24 hours.

Immobilization was accomplished by first creating a layer of glutaraldehyde bonded to the silanized electrode surface. A 2.5% glutaraldehyde solution was made with 27 mL distilled water and 3 mL of 25% glutaraldehyde. Immobilization was accomplished by immersing the wire in the glutaraldehyde solution, continuously stirred, for one hour at room temperature. The wires were then rinsed with water for one hour at room temperature, after which the electrodes were immersed in the enzyme solution. The enzyme solution was prepared with 80 mg of glucose oxidase in 2 mL phosphate buffer of pH 7.4. The wires were left in the enzyme solution overnight at 4°C to allow immobilization onto the glass layer.

COMPARISON OF DIFFERENT COATINGS FOR FIRST SENSOR PROTOTYPE

Tests with different coatings have been performed, and Table 1 shows a summary of the sensor characteristics obtained with sensors employing five different polymer coatings, namely; cellulose acetate (CA), Silastic 3-5025, Silastic Q7-2213, polyurethane (PU) and polyvinylchloride (PVC). Table 1 presents a variety of concentrations of the polymer coating solutions and the resulting parameters of the calibration curves of the sensors: linearity (as the upper limit of the calibration curve linear range), sensitivity (slope of the linear portion of the calibration

curve) and response time. It should be noted that any coated sensors showing a non-linear response were assumed to have a linear range of 2.2 mM as the linearity below this range was not investigated. It can be seen that extended linearity is not obtained with any of the five polymers below a polymer coating concentration of 5%. At a concentration of 5% of PVC and CA solutions and 7% of PU solutions a linearity of up to 8.9, 11.1 and 13.3 mM respectively was obtained. It is also noted that the use of the Silastic coatings even at very high polymer solution concentrations did not show a significant increase in the linearity, hence the use of Silastic as an outer polymer membrane was abandoned. Increasing the polymer coating solution concentrations extends the linear range of the sensor response further, but is accompanied by a corresponding decrease in the sensor sensitivity. This can be explained by assuming that increasing the polymer coating concentration produces a thicker or less porous coating. Increasing the coating thickness or decreasing its porosity limits the flux of glucose and thus decreases the amount of hydrogen peroxide produced, reducing the response to a given glucose concentration.

Table 1. Polymer coatings and their effect on the linearity, sensitivity and response time of the prepared first prototype sensors.

Coating	Concentration (mg %)	Linearity (mM)	Sensitivity (nA/mM)	Response time (secs)
Cellulose Acetate	0.2	2.2	10.8	69
	1	2.2	5.4	69
	5	11.1	3.15	120
	7.5	17.7	2.25	120
	10	26.7	1.62	183
	15	No response		
Silastic 3-5025	10	2.2	21.4	55
	20	2.2	16.7	58
	30	2.2	9.4	58
	33	6.7	4.05	60
Silastic Q7-2213	60	6.7	24.3	120
	80	15.6	4.95	120
Polyurethane	0.5	2.2	34.74	19
	1	2.2	18.9	19
	5	2.2	13.95	22
	7	13.3	4.14	22
	10	17.8	2.16	24
	12	31.1	1.35	24
	15	No response		
Polyvinylchloride	0.2	2.2	23.76	32
	1	2.2	17.57	32
	5	8.9	12.15	34
	8	17.7	2.7	35
	10	37.8	1.8	35
	12	No response		

USE OF ANTICOAGULANT

In thromboembolic conditions it is well known that it is desirable to delay the coagulation process to a certain degree. Therefore, various anticoagulants have been developed for treatment of these conditions. The ones most useful clinically are heparin and

Dicumarol.

PLASMA TESTS

In vitro plasma tests try to simulate the working environment in the body. It can be seen from the previous discussion that all three sensor prototypes responded well in plasma showing close correlation with their performance in buffer. Some decrease of sensitivity may be observed in the case of the second and third sensor prototypes. This is expected due to adsorption of proteins on the sensor surface. No decrease in sensitivity is observed for the first sensor prototype.

Table 2 shows the evaluation of the second prototype sensor performance in plasma and in buffer solution before and after the plasma test. Comparing the sensor response in buffer solution before the plasma test with that during the plasma test, it can be seen that the values of the sensor response to glucose concentration from 10 to 12 mM in both measurements coincide (within 2%). At higher glucose concentration the signal in plasma is lower than that obtained in buffer solution but with an acceptable deviation of 7%. A higher response of the sensor to glucose levels in plasma within the range 4.4 - 8.4 mM is observed. The values of the sensor response

obtained in buffer after the plasma test practically coincide with those obtained in plasma.

Table 2. Second sensor prototype response to glucose in buffer solution and in bovine plasma.

Glucose Concentration mM	Sensor Response in Buffer Solution Before Plasma Test nA	Bovine Plasma nA	Buffer Solution After Plasma Test nA
4.4	63	92	104
6.6	94	122	127
158.4	137	157	155
10.2	186	187	188
12.1	201	205	205
15.6	230	213	223

INTERFERENCE TESTS

The effect of physiological interferents on the sensor signal is also another important aspect to be considered as the sensor will be implanted in the body. The effect of interferents was tested by five common substances. The first sensor prototype shows a decrease in sensitivity after the addition of interferents. The second sensor prototype shows a decreased effect of glycine, urea and ascorbic acid. There is a marked effect of uric acid on the sensor signal as well as a decrease in sensor sensitivity. The second sensor prototype completely eliminates the effects of glycine and urea while greatly reducing the effect of uric and ascorbic acid. There is also no decrease in sensor

sensitivity as a result of adding the interferents. Similar results were obtained for the third prototype.

Interference tests have been performed, and Table 3 shows the effect of addition of carbohydrates and other common interferents. It can be seen that the response to carbohydrates (other than glucose) is negligible with respect to the sensor response to the same concentration of glucose. This indicates that the sensor signal is due to the oxidation of glucose by GOD and not the electrochemical oxidation of glucose on the platinum electrode surface. Nafion coatings caused a 50% reduction in the response of the sensor to other common interferents.

Table 3. Second sensor prototype response of non-coated and Nafion coated sensors to carbohydrates and other common interferents.

Substance	Concentration	Sensor response	
	mM	Non-coated nA	Nafion coated nA
Glucose	4.4	465	105
Sucrose	4.4	8	< 1
Fructose	4.4	6	< 1
Lactose	4.4	2	< 1
Glycine	10	35	21
Urea	5	25	17
Ascorbic Acid	6	28	14
Acetaminophen	20	420	172
Uric Acid	15	981	352

OVERALL PERFORMANCE OF PROTOTYPE SENSORS

The overall performances of the three manufactured sensor prototypes are compared in Table 4. The three sensor prototypes are based on the immobilization of glucose oxidase in a poly(1,3-phenylenediamine) film (first prototype), in a platinum black matrix (second prototype), or on Stöber glass bead (third prototype). The ultimate goal of the sensor development will be its use in intravascular glucose monitoring and especially in victims in risk of hemorrhagic shock. A further use is in monitoring glucose levels in a chemical process. Thus, a comparison of the performance of each of the three sensor prototypes and how well they satisfy the constraints set forth by these goals would be helpful in evaluating which sensor

prototype will be used.

Hemorrhagic shock is characterized by the alteration of glucose concentrations between the hypoglycemic and hyperglycemic levels. To simplify calibration and operation of the sensor, the sensor response should be linear in glucose concentration over the range of interest. This linearity should be at least 22 mM (400 mg/dL) to assure linear output of the sensor within some safety margin. It can be seen from Table 4 that all sensor prototypes satisfy this requirement. The linearity of the sensor output signal versus the glucose concentration is extended to at least 22 mM. The first sensor prototype showed a linearity of 26.7 mM with sensitivity of 1.62 nA/mM, 31.1 mM with a sensitivity of 1.35 nA/mM, and 37.7 mM with a sensitivity of 1.8 nA/mM for cellulose acetate (CA), polyurethane (PU) and polyvinylchloride (PVC) coated sensors, respectively. The second sensor prototype (with enzyme entrapped in a platinum black matrix) showed a linearity of up to 33 mM. The third sensor prototype also showed a linearity of at least 22 mM.

Another requirement for monitoring glucose levels during hemorrhagic shock is a fast sensor response (short response time) to changes

in glucose concentration. Comparing the response times of the three sensor prototypes, the first sensor prototype shows response times of 183, 24 and 35 seconds for CA, PU and PVC coated sensors, while the second sensor prototype shows a response time of 60 seconds; the third prototype shows a resonse time of 90 seconds. Another test reflecting the response times of the sensors is the in vitro hemorrhagic shock simulation test. This test reflects the response times of the sensors while responding to large changes in glucose concentration (between hypoglycemic and hyper glycemic levels). Table 4 shows the first sensor prototype having an outer PVC membrane shows a response time of 1.7 minutes for the increasing and 5.1 minutes for the decreasing step. The second sensor prototype shows a response time of 5 minutes for the increasing and 10 minutes for the decreasing step changes. It is thus seen that the first and second sensor prototypes show the shortest response times in response to both small and large changes in glucose concentration.

Table 4. A comparison of the performance of the three developed sensor prototypes with the different external polymer membranes.

Performance	First Prototype	Second Prototype	Third Prototype
Linearity	CA: 26.7		

(mM)	PU: 31.1 PVC: 37.7	33	PVC: 22
Sensitivity ($nA/mM)	CA: 1.62 PU: 1.35 PVC: 1.80	36	PVC: 0.82
Response Time (seconds)	CA: 183 PU: 24 PVC: 35	60	PVC: 90
Hemorrhagic Shock: Duration of Increasing Glucose Step (minutes)	PVC: 1.7	5	
Duration of Decreasing Glucose Step (minutes)	PVC: 1.5	10	
Signal Variation	< 5%	< 10%	
Effect of Interferents	No change in Sensitivity	Decreased Sensitivity	
Plasma Test	Decreased sensitivity	Decreased Sensitivity	No change in Sensitivity

PC: polycarbonate CA: cellulose acetate PU: polyurethane
N PC: Nafion coated polycarbonate S PC: silastic coated polycarbonate

The reproducibility of the sensor signal is good in all three sensor prototypes. The variations in the sensor response do not exceed 5% in the first prototype response, while it is less than 10% for the second prototype.

All three prototypes showed a life time of at least one week with no significant change in the sensor performance, hence satisfying the life time

requirement.

One last aspect to be considered is the ease of fabrication of the sensor prototypes. In general, the three sensor prototypes are easily fabricated. The first prototype has the advantage of electrochemical growth of the entrapping film, hence allowing better controllability on the thickness of the film and hence the amount of enzyme entrapped.

The present invention is, of course, in no way restricted to the specific disclosure of the specification, Examples and drawings, but also encompasses any modifications within the scope of the appended claims.

CLAIMS:

1. A coated wire sensor, characterized by:

a platinum wire (1) as a working electrode, said wire having a first end (1');

a sensor body (3) in the form of a hollow member disposed about at least a portion of said platinum wire (1), said sensor body (3) being spaced from said platinum wire;

a reference electrode (6) that is operatively associated with said first end (1') of said platinum wire (1) but is not in contact with said wire;

insulation (2) disposed along at least a portion of a length of said platinum wire (1) between said wire and at least one of said sensor body (3) and said reference electrode (6);

enzyme-containing material (4) disposed on said first end (1') of said platinum wire (1), said enzyme-containing material (4) comprising enzyme chemically linked to fine carbon particles; and

a coating (5) disposed over said enzyme-containing material (4).

2. A sensor according to claim 1, wherein said sensor body (3, 3B) is in the form of a hollow stainless steel member or a hollow

plastic member which can have a diameter of, for example, approximately 1 mm.

3. A sensor according to claim 2, wherein said hollow stainless steel member (3) extends to the vicinity of said first end (1') of said platinum wire (1), and said reference electrode (6) is connected to said hollow stainless steel member (3) on a side thereof remote from said insulation (2).

4. A sensor according to claim 2, wherein said reference electrode (6B) is disposed about a portion of said insulation (2B) in the vicinity of said first end (1'B) of said platinum wire (1B).

5. A sensor according to claim 1, wherein said insulation (2) is selected from the group consisting of polyurethane, polyvinylchloride, polyacrylate, Silastic, polytetrafluoroethylene, polycarbonate, and cellulose acetate, and said enzyme-containing material (4) is selected from the group consisting of enzyme incorporated in a platinum black matrix, enzyme incorporated in poly (1,3-phenylenediamine) film, enzyme incorporated in poly (1,2-phenylenediamine), enzyme incorporated in 1,2 diaminobenzene and enzyme incorporated in 1,3 diaminobenzene, and said coating (5) is a polymer

selected from the group consisting of polyurethane, polyvinylchloride, Silastic, and cellulose acetate.

6. A sensor according to claim 5, wherein an anti-clotting agent is added to said coating (5) such as heparin.

7. A sensor according to claim 1, wherein said enzyme chemically linked to fine carbon particles is in a stabilizing agent.

8. A sensor according to claim 7, wherein said stabilizing agent is selected from the group consisting of Nafion, and polyvinylalcohol.

9. A sensor according to claim 1, wherein said first end (1', 1'A) of said platinum wire (1, 1A), with said enzyme-containing material (4, 4A) and said coating (5, 5A) thereof, has a rounded or squared-off configuration.

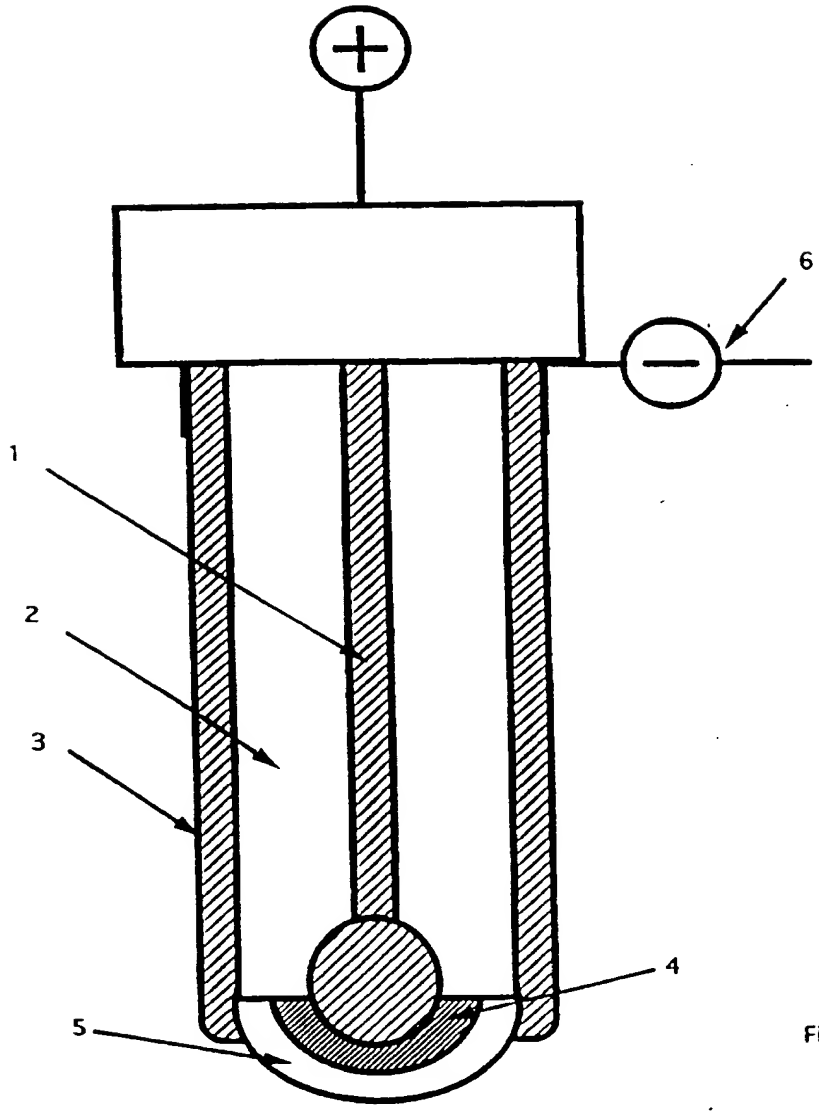

Fig. 1

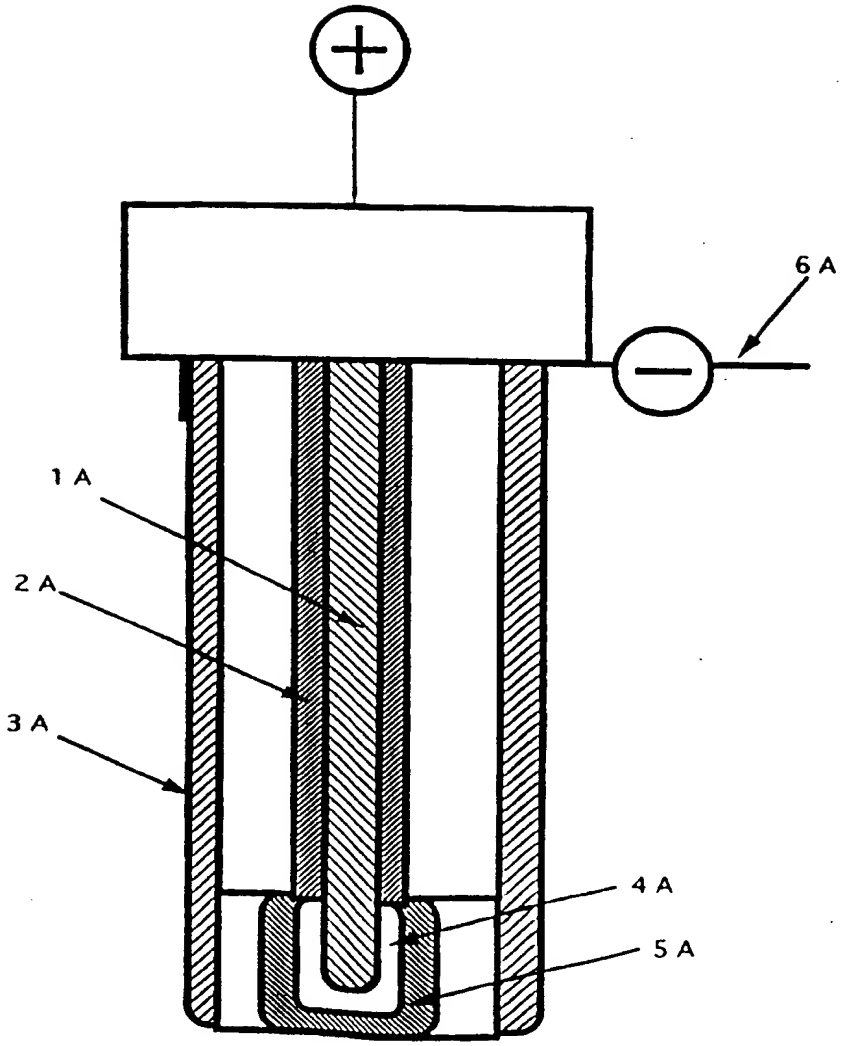

Fig. 2

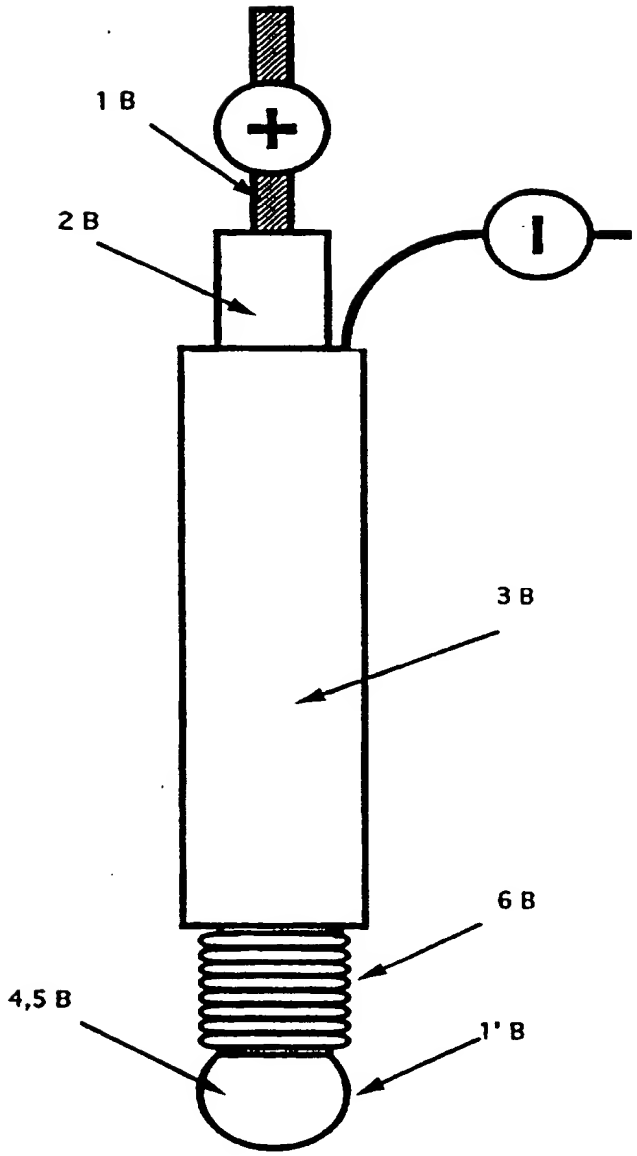

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17893

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 27/327
US CL :204/402
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 204/402, 415; 205/777.5, 778, 792

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,919,141 (ZIER ET AL) 24 April 1990, see column 4, line 54 to column 5, line 13 and column 7, line 24.	1-9
Y	US, A, 5,089,112 (SKOTHEIM ET AL) 18 February 1992, see column 5, line 34 to column 6, line 65.	1-9
Y	US, A, 5,272,087 (EL MURR ET AL), 21 December 1993, see column 2, lines 56-58 and column 5, line 40.	2-4
Y	SASSO, S.V. Electropolymerized 1,2-Diaminobenzene as a Means to Prevent Interferences and Fouling and to Stabilize Immobilized Enzyme in Electrochemical Biosensor. Anal, Chem. 1990, Vol. 62, pages 1111-1117, see the abstract.	5-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search
07 JANUARY 1997

Date of mailing of the international search report
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Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
T. TUNG
Telephone No. (703) 308-3329

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17893

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GEISE, R.J. Electropolymerized 1,3-Diaminobezene for the construction of a 1,1' - Dimethylferrocene Mediated Glucose Biosensor. Anal. Chim. Acta. 1993, Vol. 281, pages 467-471, see page 470 left column.	5-6
Y	IKARIYAMA, Y. Electrochemical Fabrication of Amperometric Microenzyme Sensor. J. Electrochem. Soc. March, 1989, Vol. 136, pages 702-706.	5-6